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THE ROLE OF PROSTAGLANDIN A IN THE CONTROL OF SODIUM HOMEOSTASIS AND BLOOD PRESSURE

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THE ROLE OF PROSTAGLANDIN A IN THE CONTROL OF SODIUM HOMEOSTASIS AND BLOOD PRESSURE

Randall Mark Zusman

B.S. Chem., University of Michigan, 1969

A Thesis Presented to the Faculty of the

Yale University School of Medicine

in Partial Fulfillment of the Requirements for the

Degree of Doctor of Medicine

New Haven, Connecticut

1973

To my family

TABLE OF CONTENTS

Page

Acknowledgements..... i

List of Tables..... ii

List of Figures iii

I. INTRODUCTION..... 1

A. The Anti-hypertensive Function of the Kidney..... 1

B. The Prostaglandins - Renal Vasodepressor Lipids..... 5

II. PURPOSE OF THIS INVESTIGATION..... 8

III. EXPERIMENTAL STUDIES..... 8

A. Radioimmunoassay of the Prostaglandins..... 8

B. The Effect of Chronic Sodium Loading and Chronic Sodium Restriction on Prostaglandin Synthesis and Release..... 24

1. Plasma Prostaglandin A, E, and F Levels in Normal Humans on High, Low, and Ad-Lib Sodium Intake Diets..... 25

2. Plasma and Renal Prostaglandin A Levels in Spontaneously Hypertensive and Normotensive Wistar Rats on High, Low, and Normal Sodium Intake Diets..... 36

C. Prostaglandin A Levels in Hypertensive Humans..... 43

IV. CONCLUSION..... 55

A. The Role of Prostaglandins in Sodium Homeostasis..... 55

B. Prostaglandin A as a Circulating Anti-hypertensive Hormone..... 60

C. The Kidney as the Source of Circulating Prostaglandin A..... 60

V. BIBLIOGRAPHY..... 62

ACKNOWLEDGEMENTS

I would like to extend my sincere gratitude to Dr. Burton V. Caldwell and Dr. Leon Speroff for their assistance in the completion of this investigation. They have given freely of their expertise in this field and their advice has been invaluable to my work. It has been a great pleasure to work in the pleasant atmosphere they have established in their laboratory.

I would also like to thank Dr. Patrick J. Mulrow for his interest in my work. He has contributed significantly to the completion of a number of my experimental studies and his critical analysis of the experimental results has been valuable in the evaluation of the data obtained.

I would like to thank Drs. Barr Forman, George Schneider, and David Spector for their assistance in the completion of a number of the experimental and clinical studies.

There are always numerous persons who by their continued presence make important contributions to any study such as this, I would therefore like to extend my gratitude to William Brock, Carlene Dowell, Genevieve Edwards, and Jon Gordon for their continued encouragement and technical assistance, and to Dawn Sassi for secretarial assistance.

LIST OF TABLES

Number	Title	Page
1	Specificity of Antiserum Directed Against PGE_2- BSA	11
2	Radioimmunoassay of Known Amounts of PGA_2 Added to Plasma	19
3	Effect of Sodium Intake on Blood Pressure, Serum Sodium, Serum Potassium, Serum Creatinine, Creatinine Clearance, Sodium Excretion, Plasma Renin Activity and Weight in Normal Humans	30
4	Effect of Sodium Intake on Plasma Prostaglandin A, E, and F Levels in Normal Humans	32
5	Effect of Sodium Intake on Plasma and Renal Prostaglandin A Concentrations in Normotensive Wistar and Spontaneously Hypertensive Aoki Rats	39
6	Plasma Prostaglandin A Concentration and Renin Activity in Patients with Essential Hypertension	49
7	Plasma Prostaglandin A Concentration and Renin Activity in Patients with Renal Artery Stenosis	50

LIST OF FIGURES

Number	Title	Page
1	Cross Reactions of Various Prostaglandins with an Antiserum to Prostaglandin A at a Dilution of 1/400	10
2	Flow Sheet for Radioimmunoassay of the A Prostaglandins	13
3	Elution Patterns of Prostaglandins A, B, E, and F via Silicic Acid Column Chromatography	14
4	Standard Curve for the Radioimmunoassay of Prostaglandin A_2	17
5	Recovery of Added PGA_2 from Plasma by Radioimmunoassay	18
6	Peripheral Plasma Levels of Prostaglandin A in Normal Males and Females, and Males with Rheumatoid Arthritis Treated with Indomethacin	21
7	Comparison of PGA Levels in Serum and Heparinized Plasma from the Same Individual	23
8	Effect of Sodium Intake on Plasma Prostaglandin A, E, and F Concentrations in Normal Humans on High, Low, and Ad-Lib Sodium Intake Diets	34
9	Effect of Sodium Intake on Daily Plasma Prostaglandin A Concentrations in Normal Humans	35
10	Plasma Prostaglandin A Levels in Hypertensive and Normotensive Rats on High, Low, and Normal Sodium Intake Diets	40
11	Renal Prostaglandin A Levels in Hypertensive and Normotensive Rats on High, Low, and Normal Sodium Intake Diets	41
12	Correlation of Plasma and Renal Prostaglandin A Levels in Hypertensive and Normotensive Rats on High, Low, and Normal Sodium Intake Diets	42

13 Comparison of Plasma Prostaglandin A Levels in Normal Humans on High, Low, and Ad-Lib Sodium Intake Diets and Patients with Essential Hypertension Associated with Renal Artery Stenosis..... 51

14 Comparison of Plasma Renin Activity and Prostaglandin A Concentration in Normotensive and Hypertensive Humans..... 52

15 A Possible Mechanism for the Control of Prostaglandin A Synthesis and Release in Response to Alterations in Sodium Intake..... 59

INTRODUCTION

THE ANTIHYPERTENSIVE FUNCTION OF THE KIDNEY

The role of the kidney in the pathogenesis of hypertensive states has been the subject of considerable discussion and controversy. In 1898, Tigerstedt and Bergman noted that saline extracts of the kidney, when injected into rabbits, elicited a pressor response. In 1940, Goldblatt demonstrated that surgical stenosis of a renal artery resulted in elevated blood pressure thereby linking the kidney with the production of hypertension. It was not until 1944, however, that Goormaghtigh, having the benefit of Ruyter's earlier description of the juxtaglomerular apparatus (1925) suggested that these juxtaglomerular cells were the source of renin, the vasopressor inducing substance. From other studies, (Kohlstaedt, Helmer and Page, 1938) it became evident that renin functioned as an enzyme acting on a component of plasma to produce the elevation of blood pressure. This renin substrate was named angiotonin by Page and Helmer (1940) and hypertensin by Braun-Menendez, et. al. (1940), in whose laboratories it was independently characterized. By agreement the name angiotensin was later adopted as the plasma component directly responsible for the increase in vasomotor tone in response to renal artery stenosis (Braun-Menendez and Page, 1958).

With continued study of the role of the kidney in regulating blood pressure it became apparent that functional renal tissue may also serve an anti-hypertensive function. Fasciolo, Houssay and Taquini (1938) and Goldblatt (1940) noted the ability of a

normally functioning kidney to blunt the rapid increase in blood pressure usually seen in response to contralateral renal artery stenosis. The blood pressure raising effect of renin given intravenously was shown to be potentiated by prior bilateral nephrectomy thus suggesting the loss of an anti-hypertensive agent produced by the kidney (Harrison, Grollman, and Williams, 1939). The administration of plasma from an animal with normally functioning kidneys was shown to reduce this sensitivity to renin in the anephric animal (Page and Helmer, 1940). Page later showed that the administration of renin to rats until the point of tachyphylaxis resulted in the release of an anti-pressor substance into the blood (Page, et.al., 1941). Numerous investigators reported that blood pressure was lowered in hypertensive and normotensive animals given saline extracts of normal renal tissue (Williams, Grollman, and Harrison, 1940; Grollman, Williams, and Harrison, 1940a, 1940b; Murphy, et.al., 1942; Stevens, 1946; Goldblatt, 1947). Characterization of the vasodepressor substance in these extracts was incomplete, however, as was the location of its intrarenal synthesis. These studies suggested that the kidney contained, and perhaps released, an anti-hypertensive agent.

The finding of hypertension in rats after bilateral nephrectomy (Braun-Menendez and Von Euler, 1947; Grollman, et.al., 1949) provided the model for further investigation of the factor responsible for the antipressor function of renal tissue. Kolff (1958) and Kolff and Page (1954) demonstrated that the perfusion of normal kidneys within the circulatory system of these renoprival hypertensive animals resulted in restoration of normal blood pressure. It was

not necessary that this kidney tissue function in excretion since anastamosis of the ureter to the inferior vena cava permitted an identical return of the blood pressure to normal (Kolff, 1958; Braun-Menendez, 1958; Muirhead and Stirman, 1958; Toth and Bartfai, 1961). The serum of such renoprival rats was found to cause venoconstriction when compared with the serum of normal animals or from rats with non-excreting functional renal tissue. It was suggested, therefore, that the renoprival animal may lack a humoral vasodilating substance produced by the kidney (Rondell, McVaugh, and Bohr, 1958).

Muirhead and colleagues in a series of elegant studies (Muirhead, et.al., 1956, 1960a, 1960b, 1960c, 1962, and 1971) showed that the renal tissue need not be structurally intact to exert its vasodilating or antihypertensive role. Using intraperitoneal or intravenous injections of fragments of whole kidneys or medullary or cortical sections of the kidneys suspended in saline, they found that after 4 days, rats without kidneys developed hypertension. Animals receiving fragments of whole kidney tissue or renal medulla, however, did not develop elevated blood pressures. Of special significance was the finding that renal cortical tissue provided no protection whatsoever from the development of elevated blood pressures. Histologic examination of the omentum and mesentary of animals which received intraperitoneal injections revealed islands of epithelial and stromal interstitial cells without evidence of viable juxtaglomerular cells.

Studies of the chemical nature of the extracts of renal tissue which produced lowering of blood pressure in various hypertensive states (Muirhead, et.al., 1960b) revealed that the active principle possessed the following characteristics:

1. Loss of activity at alkaline pH (greater than 10) for one hour (Hamilton and Grollman, 1958; Hickler, et.al., 1963; Hickler, et.al., 1964a).

2. Stable at acid pH (less than 2) for one hour (Hickler, et.al., 1964b).

3. Soluble in chloroform (Hickler, et.al., 1964b).

4. Soluble in ethanol (Muirhead, et.al., 1961; Lee, et.al., 1963).

5. Unstable at temperatures greater than 50°C (Hamilton and Grollman, 1958; and Lee, et.al., 1963).

6. Not a polypeptide, resistant to protein hydrolases, trypsin and carboxypepsidase (Muirhead, et.al., 1963; Lee, et.al., 1963; Hickler, et.al., 1964b).

7. No ultraviolet light absorption at greater than 240 millimicrons (Muirhead, et.al., 1963).

8. Not a nucleotide (Lee, et.al., 1963).

9. Not bradykinin (Hickler, et.al., 1963).

10. Polyunsaturated lipid with ketone and hydroxyl functional groups (Lee, et.al., 1964; Hickler, et.al., 1964b).

11. Not a natural free fatty acid, glyceride, or phospholipid (Hickler, et.al., 1964a, 1964b).

12. Molecular weight less than 4500 (Lee, et.al., 1962; Muirhead, et.al., 1962; Lee, et.al., 1963; Hickler, et.al., 1964b).

13. Found exclusively in the medullary tissue of the kidney (Hickler, et.al., 1964b).

The prostaglandins, fatty acids found in many tissues, share the above mentioned characteristics.

THE PROSTAGLANDINS - RENAL VASODEPRESSOR LIPIDS

In 1965, Lee and co-workers reported the isolation of 3 vasoactive lipids from renal tissue, two of these were identified as prostaglandin E_2 (PGE_2) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) both of which had been previously noted to be present in seminal fluid (von Euler, 1934). The other substance, which was a potent vasodilator, was named prostaglandin E-217, because of its chemical similarity to the E-type prostaglandins and its absorption peak at 217 millimicrons. Lee gave this substance the name "medullin" although it was later identified as a new prostaglandin and designated as prostaglandin A_2 (PGA_2). Further investigation revealed that these prostaglandins were found only in the renal medulla but not the renal cortex (Lee and Crowshaw, 1968; Horton and Jones, 1968), consistent with the previous findings on the location of the vasoactive substance(s).

The prostaglandins have been the subject of intensive investigation in the past decade, and since the entire spectrum of their chemistry and physiological actions have recently been reviewed (Weeks, 1972; Hinman, 1972), only a brief description of

some of their characteristics will be given here. It would, however, be of value to summarize those characteristics relating to the vasomotor effects of these compounds. The three major prostaglandins are named A, E, and F according to the chemical nature of the cyclopentane ring portion of the molecule. Prostaglandins of the A series are derived by the dehydration of the E series, and the B series, formed from A by rearrangement of the double bond within the cyclopentane ring. In the enzymatic metabolism of PGA, PGB is formed via an intermediate molecule, PGC, which then rearranges to yield PGB. Prostaglandins of the A and E series are vasodepressor in action when administered intraarterially, while prostaglandin F is a vasopressor; prostaglandin B has little vasoactivity (Lee, 1967). Prostaglandins of the E and F series are potent smooth muscle stimulants, while prostaglandin A has little smooth muscle stimulating activity (Lee, 1967).

Because of their vasodilating action and antagonism of the vasoconstriction produced in response to angiotensin, norepinephrine, or renal nerve stimulation it was thought that either prostaglandin A or prostaglandin E may be the circulating antihypertensive substance produced by the kidney (Lee, 1969; McClatchey and Carr, 1971; McGiff, et.al., 1970). Studies on the metabolism of prostaglandins revealed that PGA_2 was most likely to have a major circulating function since greater than 85% of PGE_2 was metabolized and inactivated during one circulation through the lungs and liver, whereas less than 30% of PGA was similarly inactivated

(McGiff, et.al., 1969b).

The survival of renal interstitial cells after renal tissue fragmentation and intraperitoneal injection led to study of these cells as the source of the antihypertensive prostaglandins. Nissen reported the presence of lipid granules in these cells (Nissen, 1968a), and furthermore, noted an increase in the number of lipid droplets after salt loading. This increase in granule number was thought to be the result of increase storage of lipid molecules with a decrease in the rate of lipid release under salt loading (Nissen, 1968b; Osvaldo, and Latta, 1966). The successful isolation and growth of these cells in tissue culture revealed prostaglandins A_2, E_2, and $F_{2\alpha}$ as components of their lipid content (Muirhead, et.al., 1972b).

It was shown by numerous investigators (Johnston, Herzog, and Lauler, 1967; Vander, 1968; Carriere, Friborg, and Guay, 1971; Martinez-Maldonade, et.al., 1972; Lee, 1972a) that infusions of prostaglandins A or E into the renal artery resulted in a marked increase in sodium, potassium and chloride excretion, glomerular filtration rate, and renal blood flow. Furthermore, it was shown by McGiff and colleagues (1970a) that the infusion of angiotensin II into the renal artery resulted in the release of prostaglandin-like material into the renal venous effluent. These observations suggested that alterations in sodium intake with resultant changes in renin-angiotensin levels may effect the synthesis and release of prostaglandins from the kidney. Lee (1972b) has suggested that prostaglandin A is a circulating anti-hypertensive natriuretic hormone.

PURPOSE OF THIS INVESTIGATION

Although a hormone-like action has been suggested for prostaglandin A it has been difficult to demonstrate such a relationship because of the lack of a rapid, and reliable method for the measurement of PGA in biological samples. The first purpose of this investigation was, therefore, to develop a radioimmunoassay for the rapid determination of PGA content in the numerous samples required for physiological studies. Once developed the assay was used to study the effect of sodium intake on plasma prostaglandin levels in man, and on plasma and renal prostaglandin levels in normal and spontaneously hypertensive rats. In an effort to establish the role of PGA in vasomotor tone plasma prostaglandin A levels in hypertensive patients were measured and a model for the regulation of blood pressure and sodium balance was developed.

EXPERIMENTAL STUDIES

RADIOIMMUNOASSAY OF THE A PROSTAGLANDINS

One of the major factors limiting the full evaluation of prostaglandins as regulators or mediators of various physiologic processes has been the lack of a simplified assay with sufficient sensitivity, precision, and specificity to allow the measurement of the different classes of prostaglandins in biological samples. Recently, radioimmunoassay procedures have been reported for the F prostaglandins (PGF) (Caldwell, et.al., 1971) which have largely

overcome these limitations. However, no such method has been reported for the A prostaglandins (PGA). Numerous protocols for the extraction, isolation, and purification of the A prostaglandins and a wide variety of methods for their quantification have been reported. These include: thin layer chromatography (Horton, and Thompson, 1964), gas liquid chromatography (Ramwell, et.al., 1968), radiogas chromatography (Hamberg, 1968), biological assay (Vane, 1969), enzymatic assay (Anggard, 1966), and ultraviolet, infrared, nuclear magnetic resonance, and mass spectroscopy (Thompson, Los, and Horton, 1970). Despite the variety of techniques it has not been possible, until this time to measure the A prostaglandins in the large numbers of samples required for clinical and laboratory studies. Therefore a radioimmunoassay for the A prostaglandins was developed using antibodies prepared by immunization of rabbits with a bovine serum albumin-prostaglandin E_2 conjugate.

Materials and Methods

Prostaglandin E_2 was covalently linked to bovine serum albumin by reaction with carbodiimide reagent as previously described for preparing similar conjugates with prostaglandin $F_{2\alpha}$ (Caldwell, et.al., 1971). Considerable conversion of the BSA-PGE to BSA-PGA probably occurred during the coupling reaction, or through metabolism in the immunized animal. A study of the relative specificity of the antiserum showed the greatest affinity for PGA_2 (Figure 1, Table 1), therefore this antiserum will be referred to as anti-PGA. Rabbits were immunized weekly (for 4 weeks) with

1 mg of the conjugate divided into 4 injections, administered subcutaneously as an emulsion with saline and Freunds Complete Adjuvant (1:1). Monthly boosters were given without the Freunds complete adjuvant. The animals were bled periodically 10-16 days after the fourth and subsequent monthly immunizations.

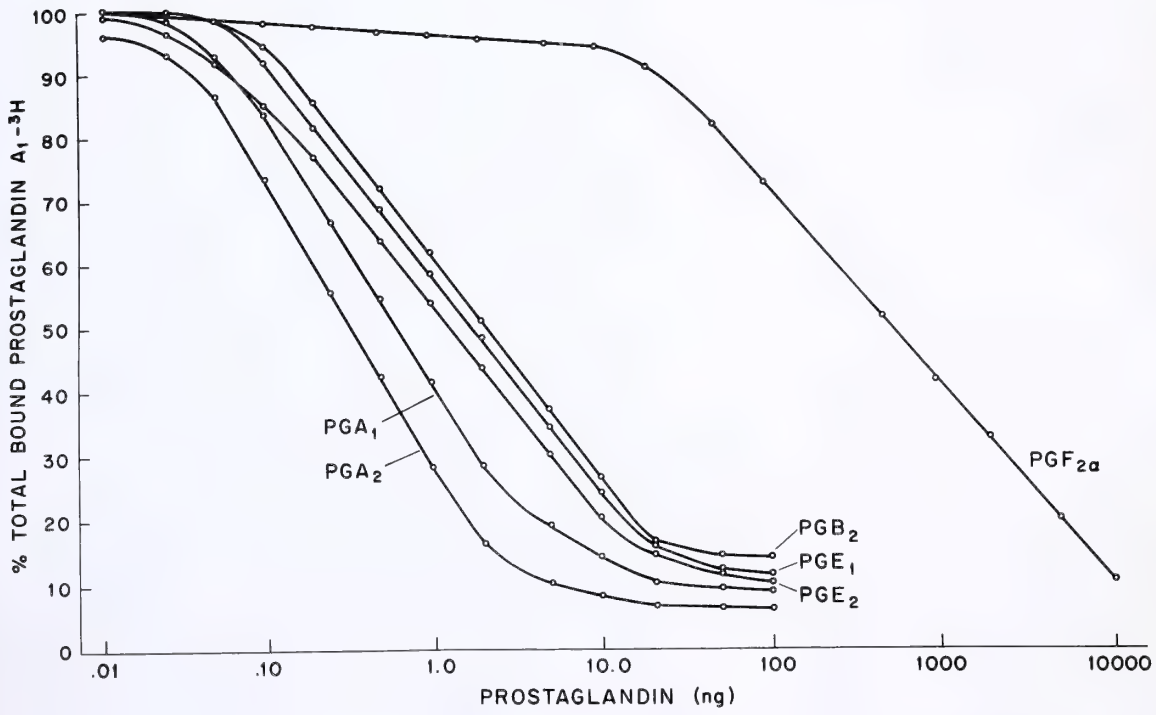
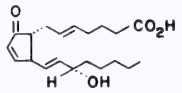
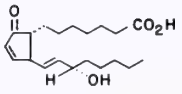
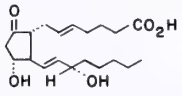
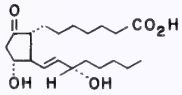
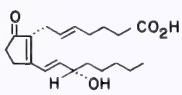
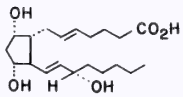


Figure 1. Cross reactions of various prostaglandins with an antiserum to prostaglandin A at a dilution of 1/400.

TABLE 1

SPECIFICITY OF ANTISERUM DIRECTED AGAINST PGE_2-BSA

PROSTAGLANDIN MEASURED	MASS REQUIRED TO DISPLACE 50% OF BOUND PGA_1-^3H	RELATIVE CROSS REACTION (%)	MOLECULAR CONFIGURATION
PGA_2	0.32 ng	100	
PGA_1	0.60 ng	53.3	
PGE_2	1.22 ng	26.2	
PGE_1	1.68 ng	19.0	
PGB_2	2.00 ng	16.0	
$PGF_{2\alpha}$	500.0 ng	0.06	

Extraction and Separation of the A Prostaglandins from Plasma

A flow sheet detailing the method is shown in Figure 2. To each 10 ml glass stoppered extraction tube, approximately 800 cpm PGA_1-^3H was added in 0.1 ml ethanol and dried under air. One ml of plasma was added to the tube and gently vortexed to ensure solubility of the trace. The sample was brought to pH 3.5 with 0.1 ml of 1 N HCL. The sample was vigorously extracted twice with 5 ml of redistilled ethyl acetate (Mallinckrodt, Analytical Reagent), and the tube centrifuged at 1,200 x g for 5 min. The ethyl acetate was removed and dried in a conical centrifuge tube. The efficiency of the extraction process was 90-95%.

The following solvent systems were prepared for chromatographic separation. In each case, spectroquality reagents were used fresh, and the ethyl acetate was redistilled to improve its purity.

Solvent I: Benzene-Ethyl Acetate (60:40)

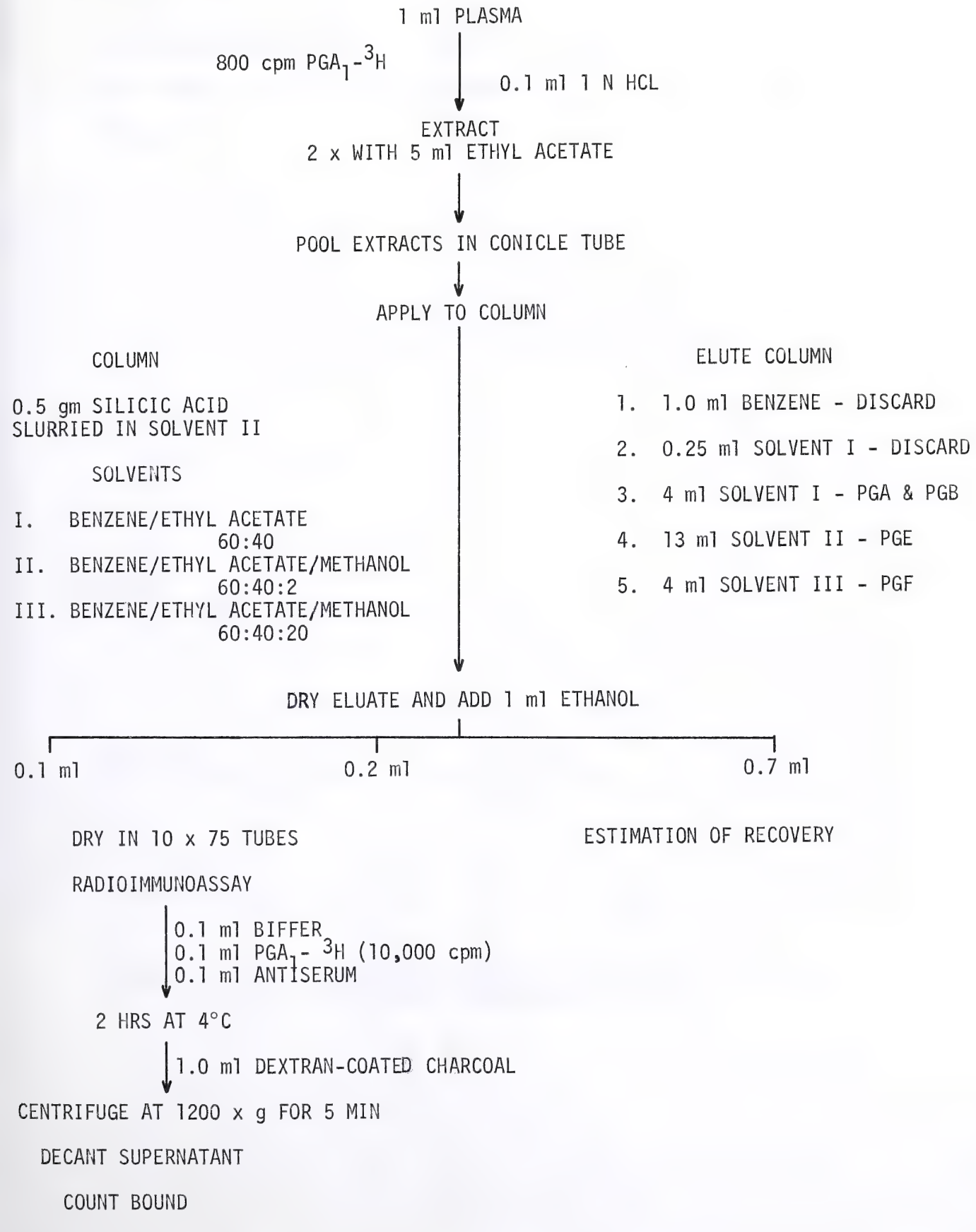
Solvent II: Benzene-Ethyl Acetate-Methanol (60:40:2)

Solvent III: Benzene-Ethyl Acetate-Methanol (60:40:20)

Brock minicolumns (1.0 x 15.0 cm glass, Macalaster-Bicknell, New Haven, Conn.) were packed with a slurry of 0.5 gms silicic acid (100 mesh, Mallinckrodt) in Solvent II. A small piece of glass fiber filter paper was inserted in the bottom of the column. Each column was washed with 5 ml of Solvent II before the sample was applied in 1.0 ml of Solvent II. Benzene (1 ml) was added and the eluate discarded, then 0.25 ml of Solvent I was added and the eluate discarded. Four ml of Solvent I was added next and the eluate contained only the PGA and prostaglandin B (PGB). The 4 ml fraction

FIGURE 2

FLOW SHEET FOR RADIOIMMUNOASSAY OF A PROSTAGLANDINS



was dried under air, and recovery of the tracer PGA_1-^3H was 65-75%. The elution pattern of prostaglandins A, B, E, and F is shown in Figure 3.

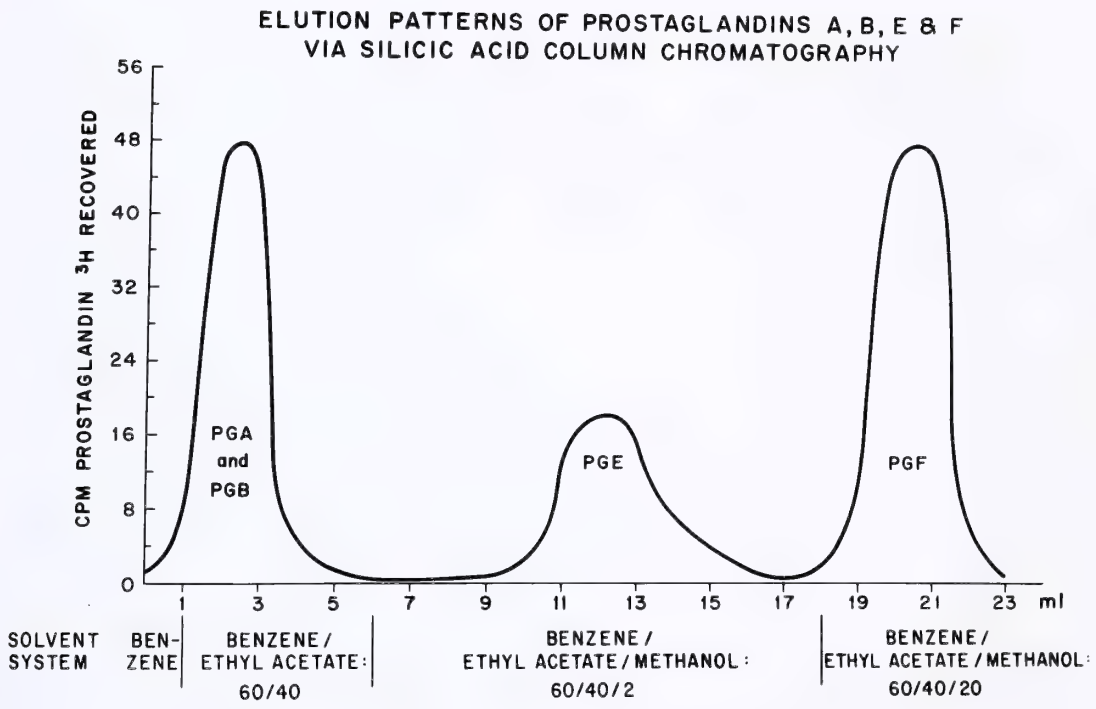


Figure 3. Elution patterns of prostaglandins A, B, E, and A via silicic acid column chromatography.

Radioimmunoassay

The assay was performed in 10 x 75 mm glass disposable tubes. Stock solutions of PGA_2 in buffer were aliquoted in duplicate through a concentration of 0.01 to 1.0 ng. The buffer was made up as follows: 5.4 g $NaH_2PO4.H_2O$, 16.4 g $Na_2HPO_4.7H_2O$, 9.0 g NaCl, 1.0 g Na Azide, 1.0 g gamma globulin, and 1000 ml distilled water. The PGA-containing fraction from the column was dried, dissolved in 1 ml of ethanol, and 0.1 and 0.2 ml aliquots were taken and dried in assay tubes. The remaining 0.7 ml was dried and counted for estimation of recovery, which ranged between 65-75%. When all sample tubes were dried, 0.1 ml of buffer was added to each tube. Antiserum (0.1 ml, 1/400 dilution in buffer) and approximately 10,000 cpm PGA_1-^3H in 0.1 ml buffer were then added to each tube to bring the final volume of all tubes to 0.3 ml. The tubes were gently vortexed and allowed to equilibrate for at least 2 hours at 4°C.

One ml of a buffer solution containing 0.25% charcoal (Norit A) and 0.025% dextran (Pharmacia, Upsala, Sweden) was added within 30 seconds to all tubes in the assay, and 2.5 minutes later the tubes were centrifuged at 1200 g for 10 minutes. The supernatant was decanted directly into a scintillation vial and 10 ml Aquasol (New England Nuclear) added. Radioactivity was determined with 30% efficiency in a Packard Scintillation Counter, Model 3375. Sample values were determined using a logit plot with linear regression analysis (Figure 4). Initial binding in the absence of any standard PGA was approximately 50%, while nonspecific binding was always

less than 3%. The equation for determination of the logit value is $l = (p/100-p)$, where p = total counts bound (sample) x 100/total counts bound (blank).

Results

A composite of 6 standard curves obtained via the described method shows a significant displacement of a sample containing 20 picograms of PGA_2 (Figure 4). When 1 ml of distilled water was carried through the method, displacement equivalent to 10-15 pg was obtained.

In order to verify the accuracy and reproducibility of the radioimmunoassay, plasma samples were obtained from patients taking indomethacin for the treatment of rheumatoid arthritis. The samples were found to have markedly reduced levels of PGA in comparison with normals, and were then considered "blank samples." The concentration of PGA in plasma of such patients (N = 7) was found to be 0.18 ± 0.16 ng/ml (mean $\pm$ SD). Known quantities of PGA_2 were added to aliquots of these pooled plasmas and the samples assayed. Table 2 shows the correlation between prostaglandin added and recovered. Linear regression analysis of this data revealed a correlation coefficient of 0.999. The equation of the "added" versus "recovered" plot is $Y = 0.99X + 0.067$. (Figure 5)

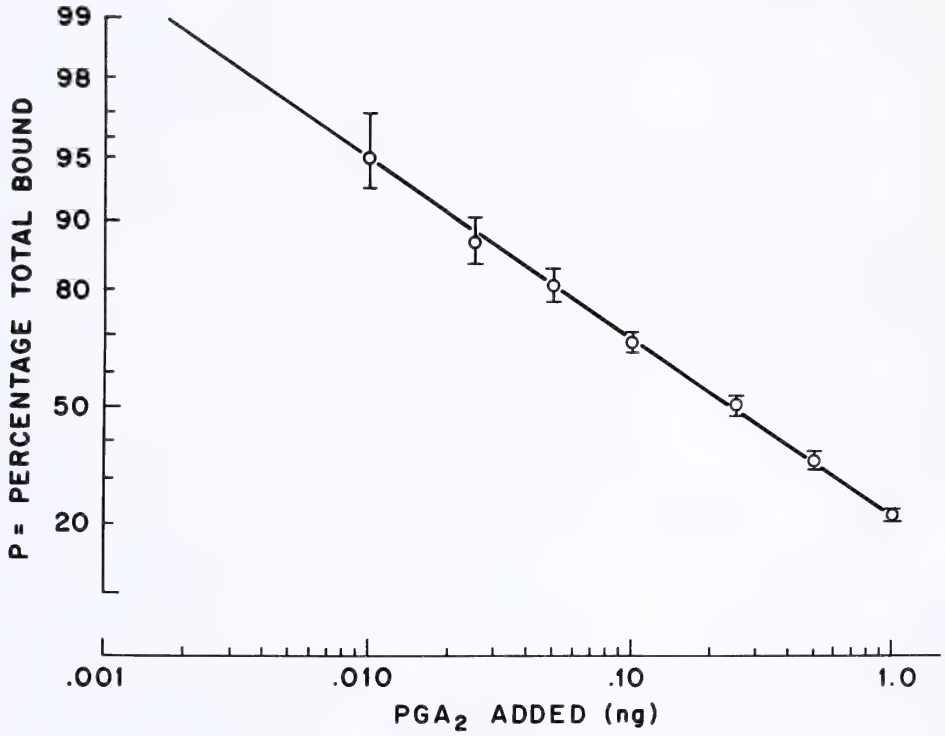


Figure 4. Standard curve for radioimmunoassay of prostaglandin A_2. Each point represents a composite of 6 standard curves (mean $\pm$ SD) run with 6 different assays.

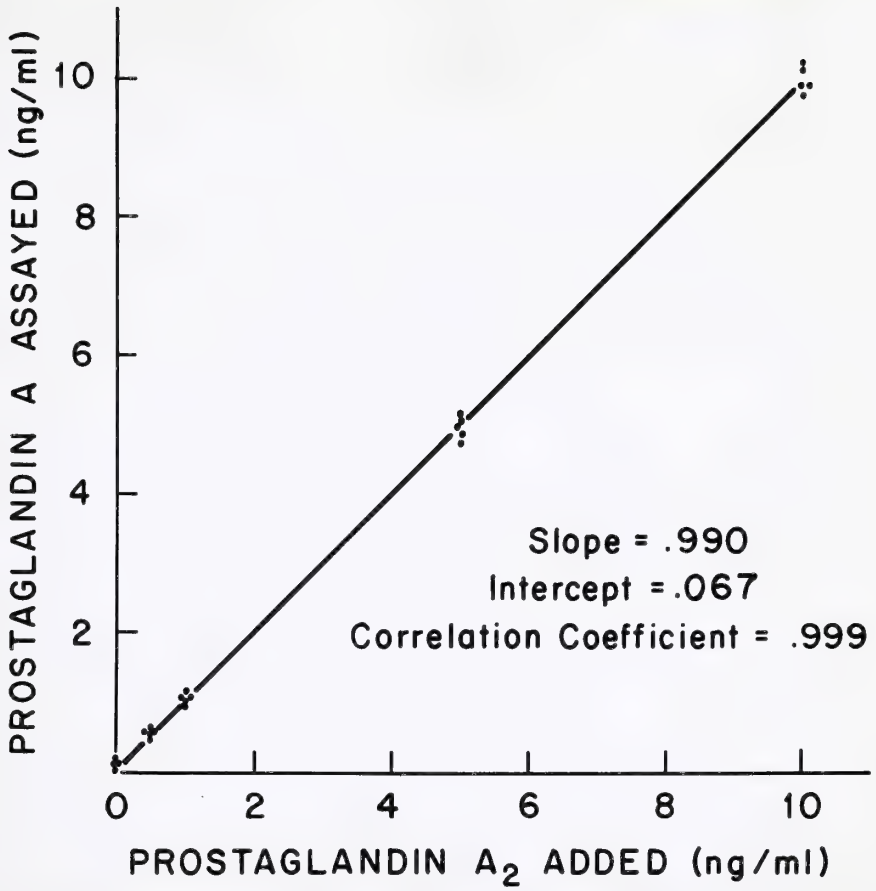


Figure 5. Recovery of added PGA_2 from plasma by radioimmunoassay.

Table 2

RADIOIMMUNOASSAY OF KNOWN AMOUNTS OF PGA_2 ADDED TO PLASMA

PGA_2 ADDED (ng)	N	PLASMA VOLUME (ml)	PLASMA LEVEL OF PGA MEASURED* MEAN ± SD (ng/ml)
0	5	1.0	0.12 ± 0.04
0.5	5	1.0	0.42 ± 0.03
1	5	1.0	1.08 ± 0.09
5	5	1.0	4.95 ± 0.31
10	5	1.0	10.00 ± 0.30
50	5	1.0	51.53 ± 1.59
100	5	1.0	96.15 ± 1.42

* corrected for recovery, uncorrected for plasma endogenous level

The results of competition studies between PGA_1-^3H and prostaglandins A_2, A_1, E_2, E_1, B_2, and $F_{2\alpha}$ are shown in Figure 1 and Table 1. Absence of the ring double bond and the presence of an hydroxyl group at position 11 was found in PGE_2 results in decreased reactivity in comparison with PGA_2. Movement of the 10, 11 double bond within the ring to the 8, 12 position as seen in PGB_2 resulted in a further decrease in reactivity in comparison with PGE_2. A consistant loss of reactivity was also related to the absence of the double bond at position 5, 6 (PGA_2 and PGE_2 vs. PGA_1 and PGE_1). Finally, a hydroxyl function at position 9 in the saturated ring results in the

most marked reduction of the cross reactivity ($PGF_{2\alpha}$ in comparison with all above prostaglandins).

Since the silicic acid chromatography completely separated the E and F prostaglandins from the A prostaglandins there is little concern with the cross reaction of these substances. PGB, however, is not separated from PGA in this system. The lack of prostaglandin isomerase in human plasma would suggest that sample contamination with PGB is of little consequence in humans (Polet and Levine, 1971). This PGB cross reactivity, however, may be of concern in samples from animals with high levels of prostaglandin A isomerase (Jones, 1970; Horton, et.al., 1971).

Initial studies of prostaglandin A in peripheral plasma of normal adults revealed concentrations of 1.39 $\pm$ 0.55 ng/ml in males and 1.62 $\pm$ 0.52 ng/ml in females (mean $\pm$ SD); this difference is not statistically significant. Patients with rheumatoid arthritis being treated with indomethacin were found to have concentrations of 0.18 $\pm$ 0.16 ng/ml; this is a significant reduction in PGA concentration ($p < 0.001$) in comparison with normal adults. (Figure 6) Further studies in 50 normal humans revealed plasma prostaglandin A levels to be 1.51 $\pm$ 0.24 ng/ml (mean $\pm$ SD), range 1.04 - 1.87. Plasma PGA levels in six anephric humans were found to be 0.19 $\pm$ 0.05 ng/ml (mean $\pm$ SD), suggesting that the kidney was a major source of circulating PGA.

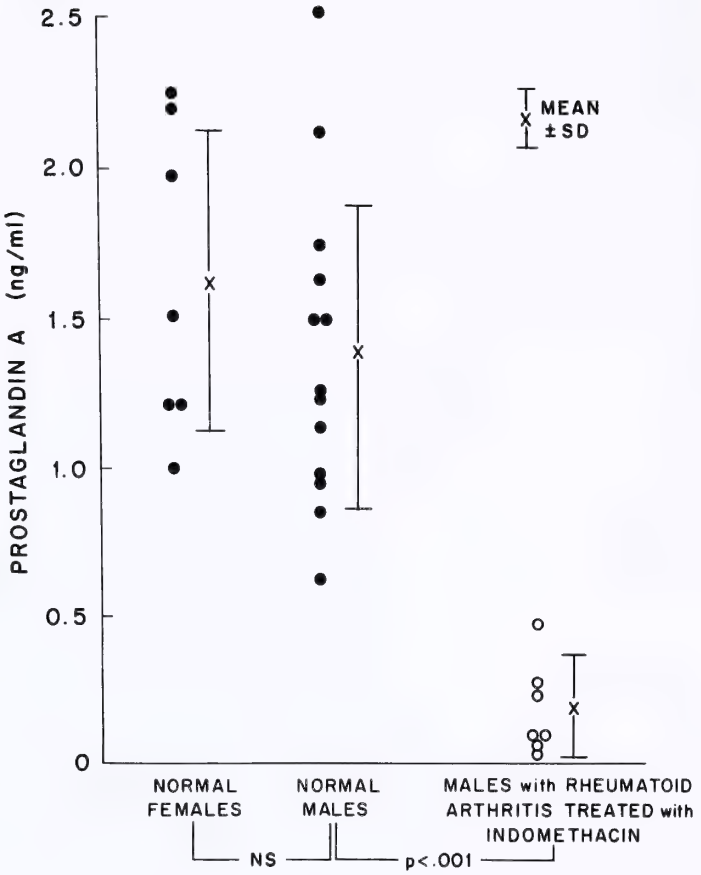


Figure 6. Peripheral plasma levels of prostaglandin A in normal males and females and males with rheumatoid arthritis treated with indomethacin.

Because of the lack of uniformity in the methods for collection of samples for prostaglandin determination, both plasma and serum samples were studied in 9 individuals. The results (Figure 7) show that there is no consistant alteration in the PGA concentration during the clotting process. The level in heparinized plasma was found to be 1.15 $\pm$ 0.40 ng/ml and in serum was found to be 1.34 $\pm$ 0.59 ng/ml (mean $\pm$ SD). In order to ensure accurate determination of PGA levels in biological samples, assays should also be completed as soon as possible after sample collection.

Discussion

Ever since the report by von Euler (1934) of the isolation of vasoactive lipid molecules from seminal vesicle tissue, attempts have been made to develop rapid, accurate, and sensitive means to measure prostaglandin content in biological samples. The radioimmunoassay procedure described in this report now allows for the measurement of prostaglandin A levels in plasma or serum samples.

In view of the relatively high levels of PGA found in human plasma in this study, and the failure of the pulmonary or hepatic circulation to inactivate prostaglandins of the A series to the same degree as PGE and PGF, Lee's postulation of a potential function for PGA as a circulating vasodilatory hormone may be justified.

Indomethacin is a known prostaglandin synthesis inhibitor (Collier, 1971; Ferriera, Moncada, and Vane, 1971; Vane, 1971; Smith and Willis, 1971), and treatment of patients with indomethacin on a long term basis for the management of rheumatoid arthritis has been shown in this study to markedly reduce the peripheral levels of PGA.

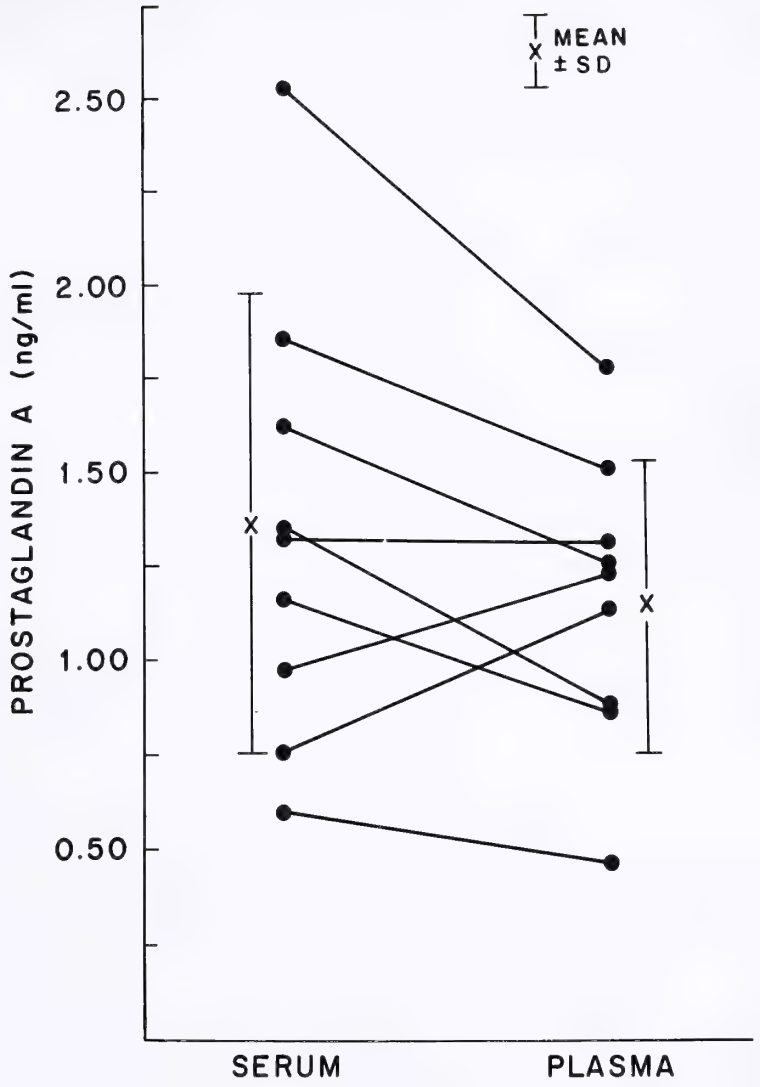


Figure 7. Comparison of PGA levels in serum and heparinized plasma from the same individual.

THE EFFECT OF CHRONIC SODIUM LOADING AND CHRONIC SODIUM RESTRICTION ON PROSTAGLANDIN SYNTHESIS AND RELEASE

In 1965, Lee and co-workers presented evidence that prostaglandin E_2 (PGE_2), and medullin, later renamed prostaglandin A_2 (PGA_2) were present in the renal medulla, the portion of the kidney known to have vasodepressor properties (Muirhead, Stinman, and Jones, 1960c). Nissen (1968a, 1968b) demonstrated that a period of salt repletion in salt depleted rats induced an increase in the number of lipid droplets in renal medullary interstitial cells. By use of tissue culture techniques it was shown that these renal interstitial cells contain prostaglandins A_2, E_2, and $F_{2\alpha}$ (Muirhead, et.al., 1972b). In addition to the presence of prostaglandins in the renal medulla increased levels of prostaglandin-like material have been demonstrated in renal venous blood in response to a number of physiologic stimuli, including renal nerve stimulation (Dunham and Zimmerman, 1970), adrenaline (McGiff, et.al., 1972), bradykinin (Lonigro, et.al., 1971), angiotensin II (McGiff, et.al., 1970a), and renal ischemia (McGiff, et.al., 1970e).

The evidence that prostaglandins were present in the renal medulla and seemed to respond to physiologic stimuli led workers to study the effect of prostaglandins on blood pressure and renal function. Prostaglandins E and A have been shown to increase renal blood flow, urine volume, free water clearance, and sodium excretion when infused in dogs (Johnston, Herzog, and Lauler, 1967; Vander, 1968; Carriere, Friborg, and Guay, 1971; Martinez-Maldonada, et.al., 1972; Lee, 1972a). Renal effects in hypertensive man are similar to those in dogs when PGA is infused at low concentrations, however,

at higher concentrations a vasodepressor effect predominates (Carr, 1970; Westura, et.al., 1970; Lee, Johnson, Smith, and Hatch, 1972). In addition recent studies in man have indicated that PGA increases aldosterone secretion independent of changes in ACTH, renin, and serum electrolytes (Fichman, et.al., 1972).

Thus a growing body of evidence suggests that prostaglandins play a role in the regulation of blood pressure and salt and water balance. Using the recently developed radioimmunoassay for prostaglandins the effect of sodium loading and restriction was studied in normal humans, and in spontaneously hypertensive and normotensive Wistar rats.

PLASMA PROSTAGLANDIN A, E, AND F LEVELS IN NORMAL HUMANS

Materials and Methods

Experiments were carried out in seven human volunteers from whom informed consent was obtained, using a protocol approved by the Clinical Investigation Committee of the Yale University School of Medicine.

There were four male and three female volunteers ranging in age from 22 to 36 years old. Drugs were not taken during the course of the study with the exception of an estrogen-progestin oral contraceptive by K.J. None of the subjects had history of serious illnesses of any type. One subject (JG) had a questionably enlarged liver; his liver function studies were normal.

The course of the experiments lasted fourteen days during which each subject went on three diets: 1) a four day ad-lib dietary control period during which the subject ingested his usual daily

sodium intake, 2) a five day duration ad-lib diet supplemented by six one gram sodium chloride tablets per day, which was calculated to yield a total of over 200 mEq sodium intake per day and 3) five days of 10 mEq sodium diet prepared by the metabolic dietary kitchen of the Clinical Research Center. In one group of subjects the low sodium diet followed the high sodium diet after a one day interval (subjects RZ, JG, DS, KJ), in the second group after a seven day interval (subjects BK, GD, GS). Potassium and caloric intake were not controlled. For comparison to control (ad-lib diet) data, only data from the last three days of each experimental period were used for calculation of mean prostaglandin levels. "P" values were determined by the student t test.

Blood pressure, weights and blood samples for prostaglandin and renin levels were taken daily between 8 and 9 A.M. Blood pressures were taken in the sitting position. Blood samples for determination of creatinine, electrolytes and hematocrit were obtained on the last two days of each experimental diet. Twenty-four hour urine collections were made during the last two days of each experimental diet period for measurement of sodium, potassium, and creatinine excretion.

Blood for renin determinations was collected in vacutainers containing EDTA, blood for prostaglandin in heparinized vacutainers. Blood was centrifuged (1200 x g) immediately at 4°C, and after the red cells were removed, plasma was stored at -20°C until analysis. Sodium and potassium were determined by flame photometry. Creatinine was measured on a Technicon Autoanalyzer. Plasma renin activity was

measured by radioimmunoassay (Haber, et. al., 1969).

Prostaglandins were determined by radioimmunoassay. Since this method does not distinguish between prostaglandins A_1, E_1 and E_2, or $F_{1\alpha}$ and $F_{2\alpha}$, the concentrations reported are of the total prostaglandins for each type and are designated simply as PGA, PGE, and PGF, respectively. Prostaglandin A cannot be separated from prostaglandin B by column chromatography. The low cross reactivity of the antiserum with PGB in comparison with PGA and the lack of an enzyme system in humans to convert PGA to PGB in the plasma indicates that contamination of the sample with prostaglandin B is of minor concern.

Results

A summary of measurements of blood pressure, serum sodium, serum potassium, serum creatinine, creatinine clearance, sodium excretion, weight, and plasma renin activity is shown in Table 3.

High Salt Diet

The high salt diet led to an increase in weight in all subjects except GS. The mean increase for the group was 1.1% of control body weight ($p < .04$). This was associated with increased urinary sodium excretion from an average baseline of 151 mEq/day to an average of 230 mEq/day. There was no significant change in blood pressure or in serum sodium or potassium concentrations. Although serum creatinine did not change significantly, creatinine clearance increased from 117.8 cc/min. to 130.7 cc/min. ($p < 0.03$).

As expected, plasma renin activity was lower during the high salt diet than during ad-lib or low salt diet in every subject.

Low Salt Intake

The 10 mEq sodium diet led to a mean weight loss of 3.7%. Systolic, diastolic and mean blood pressures fell in every individual, averaging 4.5, 3.4 and 3.7% decrease from baseline values respectively. Mean serum sodium fell 2.3 mEq/L, ($p < 0.002$) but serum potassium did not change from control levels. Every subject reached urinary sodium outputs of less than 10 mEq/day by the fifth low sodium day except GS who excreted 21 mEq/24 hours. Mean serum creatinine rose significantly from 0.96 to 1.15 mg% ($p < 0.02$). Renin levels rose over baseline values in every subject during salt restriction.

Prostaglandin A

Mean plasma concentrations of Prostaglandin A for four ad-lib control days and for the last three days of each experimental period are shown in Table 4 and Figure 8.

During the ad-lib sodium intake mean plasma PGA was 1.60 ng/ml. The level fell almost 50% during the high sodium diet to a mean of 0.82 ng/ml. During the low sodium diet PGA levels rose approximately 34% over baseline, and 161% over high sodium intake to 2.15 ng/ml. These changes were significant at $p < 0.001$.

Sequential changes of PGA are shown on a daily basis in Figure 9. In every subject PGA levels dropped dramatically during the second day of the high salt diet and plateaued at this new level. The effect of low sodium intake of PGA levels is somewhat more gradual, although this could reflect the effect of the prior salt load. However, there was no difference in the rate of change of PGA in subjects in whom low salt diet immediately followed high salt diets and in those

subjects in whom the two dietary periods were separated by six days. If prior salt loading had an effect on PGA levels during sodium deprivation, one would expect a difference in the two groups of subjects since it would seem likely that at least in the second group, control conditions would have been re-established.

Plasma PGA levels rose during the low sodium diet and plateaued during the fourth day in most subjects, although the mean for the dietary period was determined from the last three days for statistical purposes. In one subject (DS) the levels of PGA did not rise significantly for the period as a whole. We cannot ascribe this response to dietary indiscretion since other parameters changed in expected fashion during this period in this subject.

Prostaglandin E and F

Neither prostaglandin E nor F changed significantly during the dietary periods (Figure 8).

TABLE 3

EFFECT OF SODIUM INTAKE ON BLOOD PRESSURE, SERUM SODIUM, SERUM POTASSIUM, SERUM CREATININE, CREATININE CLEARANCE, SODIUM EXCRETION, PLASMA RENIN ACTIVITY, AND WEIGHT IN NORMAL HUMANS

	Systolic BP, mm HG			Diastolic BP, mm HG		
Salt Intake	Ad Lib	High	Low	Ad Lib	High	Low
Mean ± SEM	115 ± 4	121 ± 4	111 ± 4	79 ± 2	78 ± 2	76 ± 2
% Change ± SEM		+5.4 ± 2.5	-4.5 ± 1.4		-1.1 ± 1.8	-3.4 ± 0.9
P Value		NS	< .02		NS	< .01

	Serum Sodium, mEq/l			Serum Potassium, mEq/l		
Salt Intake	Ad Lib	High	Low	Ad Lib	High	Low
Mean ± SEM	139.3 ± .3	138.3 ± .6	137.0 ± .4	3.97 ± .08	4.10 ± .07	3.97 ± .09
% Change ± SEM		-0.3 ± 0.3	-1.7 ± 0.3		+3.4 ± 1.5	+0.0 ± 0.8
P Value		NS	< .002		NS	NS

	Mean BP, mm HG			Serum Creatinine, mg%		
Salt Intake	Ad Lib	High	Low	Ad Lib	High	Low
Mean ± SEM	96 ± 3	100 ± 3	93 ± 3	0.96 ± 0.5	0.95 ± .05	1.15 ± .08
% Change ± SEM		+3.8 ± 1.8	-3.7 ± 0.7		-1.6 ± 0.9	+21.3 ± 6.7
P Value		NS	< .002		NS	< .02

Salt Intake	Creatinine Clearance, ml/min			Urinary Sodium, mEq/24 hr.		
	Ad Lib	High	Low	Ad Lib	High	Low
Mean ± SEM	117.8 ± 11.3	130.7 ± 9.7	97.8 ± 10.6	151.3 ± 15	230.9 ± 10.1	10.4 ± 2.0
% Change ± SEM		+13.5 ± 4.6	-17.1 ± 5.2		+59.1 ± 12.2	-91.6 ± 2.3
P Value		< .03	< .02		< .005	< .001

Salt Intake	Weight, pounds			Plasma Renin Activity, ng/ml/hour		
	Ad Lib	High	Low	Ad Lib	High	Low
Mean ± SEM	151.6 ± 13.1	157.2 ± 13.2	149.7 ± 12.6	1.49 ± .12	.61 ±.03	8.49 ± 0.75
% Change ± SEM		1.1 ± 0.4	3.7 ± 0.5		-58.4 ± 2.3	+455.8 ± 60.8
P Value		< .04	< .001		< .001	< .001

* Mean of weight, renin and blood pressure for each person represents the average of measurements during 4 days of ad lib diet, and the final 3 days of the high and low sodium intake periods. Mean of creatinine clearance, urinary sodium, plasma sodium, plasma potassium, serum creatinine represents the average of measurements taken on the last 2 days of each dietary period.

TABLE 4

EFFECT OF SODIUM INTAKE ON PLASMA PROSTAGLANDIN A, E, AND F LEVELS IN NORMAL HUMANS

Subject	Salt Intake	PROSTAGLANDIN A ng/ml			PROSTAGLANDIN E ng/ml		
		Ad Lib	High	Low	Ad Lib	High	Low
BK		1.73	0.99	2.37	.20	.24	.25
DS		1.60	1.00	1.72	.32	.27	.22
GD		1.35	0.82	1.90	.31	.22	.20
GS		1.71	0.86	2.27	.21	.22	.31
JG		1.75	0.74	2.13	.23	.21	.27
KJ		1.61	0.64	2.20	.22	.26	.28
RZ		1.44	0.71	2.36	.29	.23	.25
Mean ± SEM		1.60 ± .06	.82 ± .05	2.14 ± .09	.25 ± .02	.24 ± .01	.24 ± .01
Mean % Δ ± SEM			-48.3 ± 3.3	+34.3 ± 6.5		-4.3 ± 7.2	+5.2 ± 12.1
P Value			< .001	< .001		NS	NS

PROSTAGLANDIN F
ng/ml

Subject	Salt Intake	Ad Lib	High	Low
BK		.36	.47	.36
DS		.32	.35	.44
GD		.29	.25	.30
GS		.35	.28	.49
JG		.44	.32	.31
KJ		.40	.31	.42
RZ		.48	.50	.39
Mean ± SEM		.38 ± .03	.35 ± .04	.39 ± .03
Mean % Δ ± SEM			-5.6 ± 8.0	5.4 ± 9.8
P Value			NS	NS

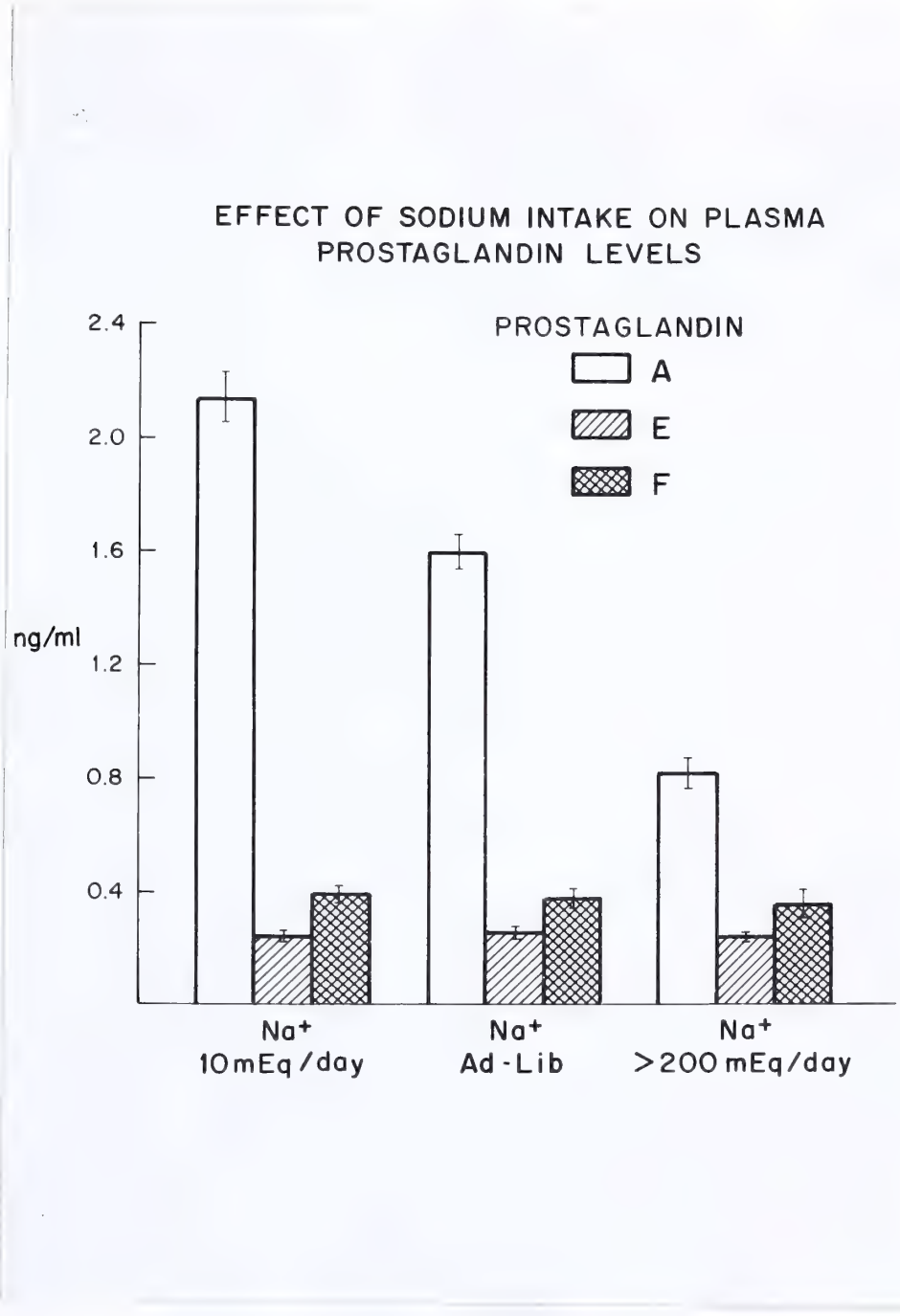


Figure 8. Effect of sodium intake on plasma prostaglandin A, E, and F concentrations in normal humans on high, low, and ad-lib sodium intake diets.

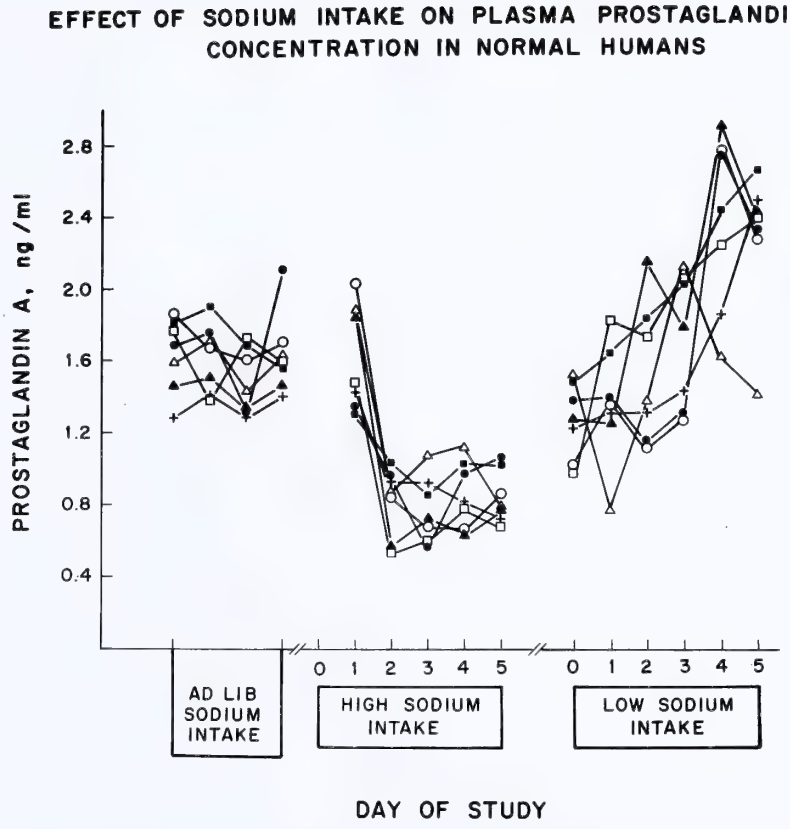


Figure 9. Effect of sodium intake on daily plasma prostaglandin A concentrations in normal humans. Day-to-day levels of prostaglandin A in peripheral plasma of seven normal human volunteers on high, low, and ad-lib sodium intake diets.

PLASMA AND RENAL PROSTAGLANDIN A LEVELS IN SPONTANEOUSLY HYPERTENSIVE AND NORMOTENSIVE WISTAR RATS

Materials and Methods

Normotensive and spontaneously hypertensive wistar rats (Okamoto and Aoki, 1963) weighing 330-380 grams, were placed on high, low, and normal sodium diets. The composition of the diets has been described (Manitus, et.al., 1960). The three diets were: 1. Normal sodium intake - a diet containing 102 mEq sodium per kilogram; distilled water was used for drinking, 2. Low sodium intake - a diet containing no measurable sodium content; distilled water was used for drinking, 3. High sodium intake - a diet containing 102 mEq sodium/kg and 0.9% sodium chloride was the drinking solution. Rat weight, food intake, and blood pressure was monitored on each sodium intake diet.

After two weeks on one of the above diets, the animals were sacrificed by decapitation, and blood collected using sodium EDTA as the anticoagulant, centrifuged (1200 X g at 4°C), and the plasma stored at -20°C. The kidneys were removed, cleaned of fat, and weighed; each kidney was homogenized in ten milliliters of saline. $^{3}H\text{-}PGA_{1}$ (New England Nuclear, specific activity 50-60 microcuries/millimole) was added to allow for calculation of total PGA recovery after homogenization and assay.

Prostaglandin A was determined by radioimmunoassay. The blood pressures were determined by the tail-cuff method in unanesthetized rats.

Results

The results of plasma and renal PGA determinations are shown in Table 5, and Figures 10 and 11. Student's t test was used for statistical analysis.

High salt suppressed and low salt increased plasma and renal PGA concentrations significantly in both normal and spontaneously hypertensive rats.

In the normotensive Wistar rats, plasma PGA levels on the high and low sodium diets differed significantly from the levels found in rats on the normal sodium intake ($p < 0.001$). Renal tissue PGA levels were also significantly altered on low and high sodium diets in comparison with normal sodium intake ($p < 0.005$ and 0.001 respectively).

In the spontaneously hypertensive Aoki rats plasma PGA levels on the low and high sodium diets differed significantly from the levels found in these hypertensive rats on the normal sodium diet ($p < 0.02$ and 0.04 respectively). Renal tissue PGA levels were also significantly altered on low and high sodium diets in comparison with normal sodium intake ($p < 0.01$ and 0.002 respectively).

Moreover plasma PGA levels were significantly higher in the spontaneously hypertensive rats in comparison with the normotensive rats on each diet, p values were less than 0.005, 0.02, and 0.001 on the low, normal, and high sodium diets respectively. Similarly renal PGA levels were higher in the spontaneously hypertensive rats, p less than 0.02, 0.04, and 0.004 on the low, normal and high sodium

diets respectively.

Linear regression analysis (Figure 3) of plasma versus renal PGA levels revealed a very high degree of correlation (regression coefficient = 0.891, p less than 0.001) in both the spontaneously hypertensive and normotensive rats.

Blood pressure did not significantly change in any of the rats on the three sodium intake diets.

There was no significant difference in the food intake of the rats on the high, low, and normal sodium intake diets. The rats drinking 0.9% NaCl took in 20-70 ml/day with a mean of 40 ml/day, resulting in a mean additional intake of 6 mEq of sodium per day. There was no significant difference in the rate of weight gain or blood pressure of any of the rat groups.

TABLE 5

EFFECT OF SODIUM INTAKE ON PLASMA AND RENAL PROSTAGLANDIN A CONCENTRATIONS IN NORMOTENSIVE WISTAR AND SPONTANEOUSLY HYPERTENSIVE AOKI RATS

	Normotensive Wistar Rats		Spontaneously Hypertensive Aoki Rats	
Dietary Sodium Content*	Plasma PGA ng/ml (Mean ± SEM)	Renal PGA ng/g (Mean ± SEM)	Plasma PGA ng/ml (Mean ± SEM)	Renal PGA ng/g (Mean ± SEM)
Low	1.69 ± 0.07 (n=6)	100.6 ± 8.5 (n=6)	3.92 ± 0.58 (n=6)	181.7 ± 26.5 (n=6)
Normal	1.00 ± 0.05 (n=6)	61.4 ± 6.4 (n=6)	1.88 ± 0.32 (n=6)	88.2 ± 9.1 (n=6)
High	0.51 ± 0.04 (n=6)	23.0 ± 2.6 (n=6)	0.91 ± 0.03 (n=4)	43.8 ± 4.7 (n=6)

* Low: no sodium in diet, Normal: 102 mEq Sodium/kg diet, distilled water to drink, High: 102 mEq Sodium/kg diet, 0.9% Sodium Chloride to drink (mean intake 40 ml/day, range 20-70)

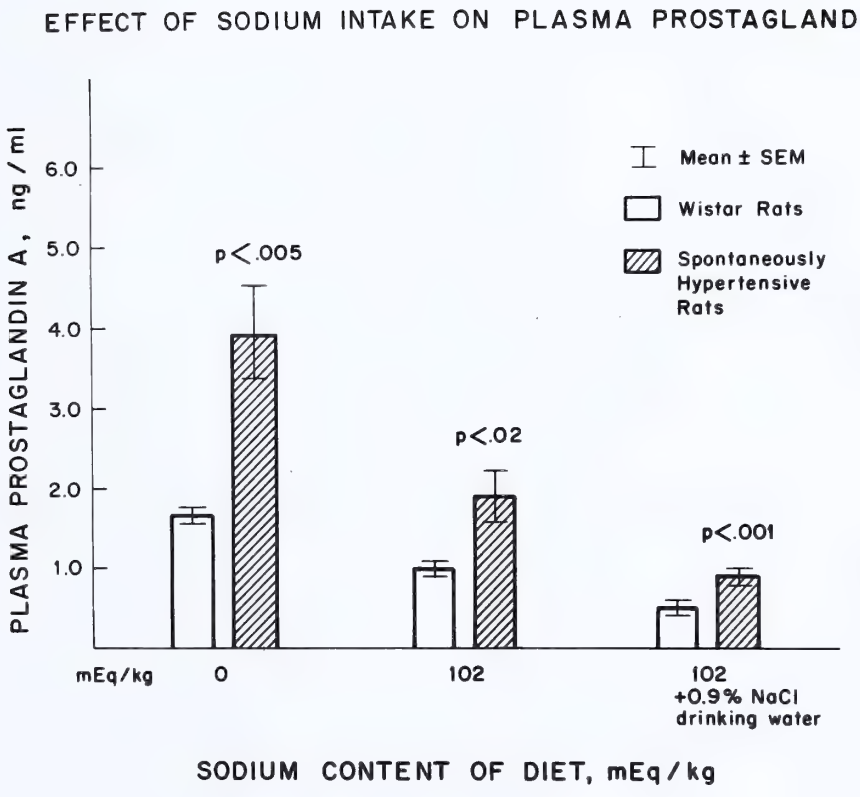


Figure 10. Plasma prostaglandin A levels in hypertensive and normotensive rats on high, low, and normal sodium intake diets

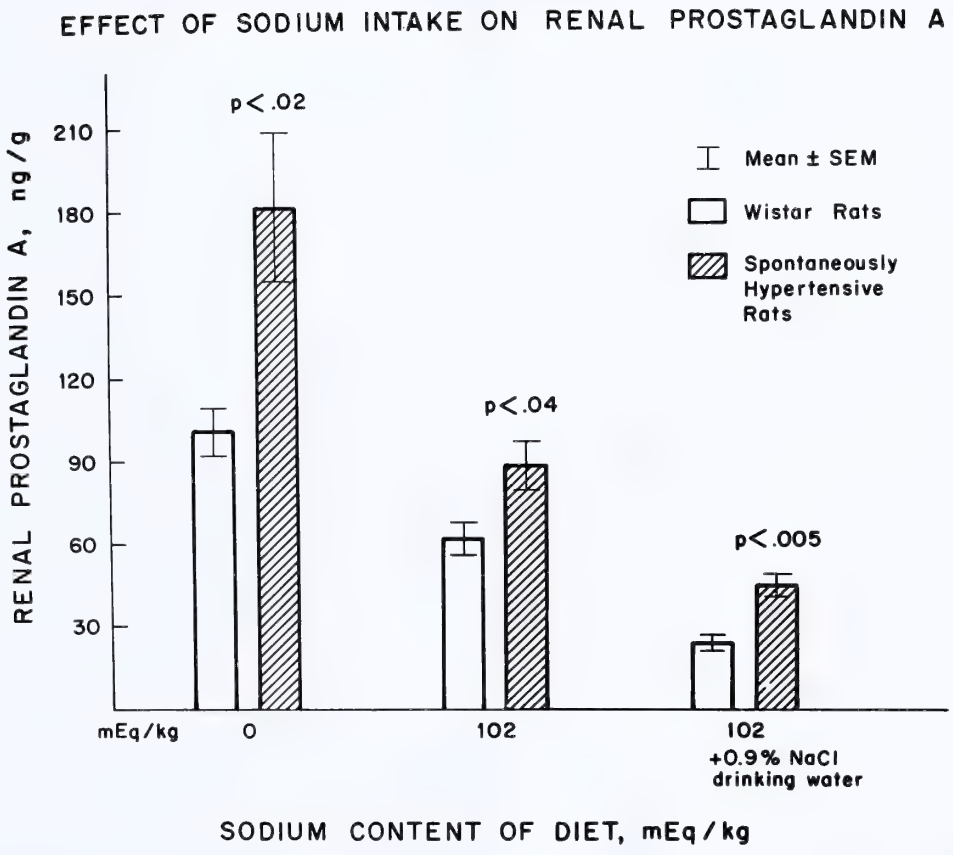


Figure 11. Renal prostaglandin A levels in hypertensive and normotensive rats on high, low, and normal sodium intake diets.

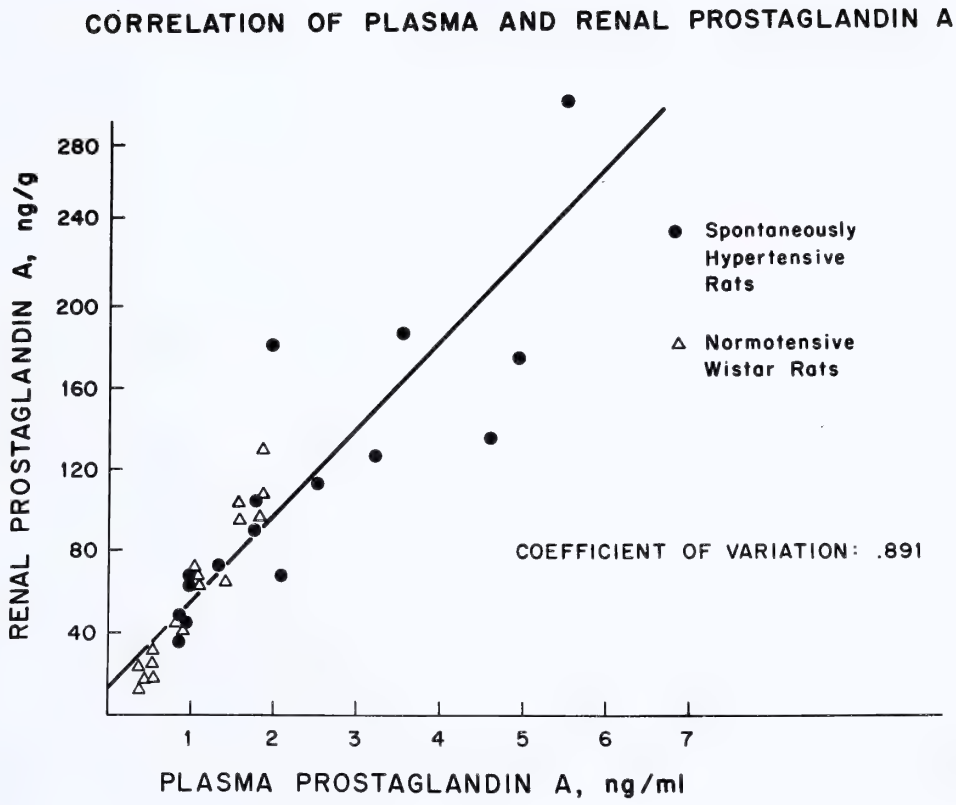


Figure 12. Correlation of plasma and renal prostaglandin A levels in hypertensive and normotensive rats on high, low, and normal sodium intake diets.

DISCUSSION

The results of these studies show that in both the normal human and the spontaneously hypertensive or normotensive Wistar rat changes in dietary sodium intake influence plasma PGA levels. In the normal human it was shown that there are no changes in circulating PGE or PGF levels. This observation does not however, exclude the possibility of renal changes in PGE or PGF since metabolism of the E and F prostaglandins by the pulmonary circulation would prevent observation of such changes in the peripheral plasma. Furthermore, the observation in the rats that the plasma changes in PGA levels are parallelled by changes within the renal parenchyma suggest that both PGA synthesis and release is increased by sodium restriction and decreased by sodium loading.

These experiments do not elucidate the source of circulating prostaglandin A, however, it seems likely that the kidney contributes to the plasma levels. Prostaglandins A, E, and F have been found in the renal medulla and prostaglandin-like substances have been shown to be released from the kidney in response to several types of physiologic stimuli. The close correlation (regression coefficient = 0.891, p less than 0.001) observed between the plasma and renal PGA levels in the rat studies certainly suggests that plasma PGA levels are effected by changes in renal PGA synthesis and release.

It is significant to note that the plasma and renal concentrations of PGA in the spontaneously hypertensive rats were higher than in

the normotensive Wistar rats in each dietary group. It is possible that PGA, a potent vasodilator, is released as a compensatory response in the spontaneously hypertensive rat. It is also possible that intrarenal stimuli, perhaps vascular spasm or local ischemia, stimulate PGA production.

PROSTAGLANDIN A LEVELS IN HYPERTENSIVE HUMANS

The evidence that the kidney plays an antihypertensive function and that prostaglandins may play an important role in protecting the "normal" blood pressure has been reviewed previously in the introduction to this thesis. Studies in the spontaneously hypertensive rat have shown that these animals have increased plasma and renal levels of prostaglandin A suggesting that there is a compensatory release of this potent vasodilator in an attempt to lower blood pressure from its abnormal elevation.

In studies by Westura et.al. (1970) and Lee, et.al. (1971) it was shown that the intravenous administration of prostaglandin A_1 to patients with essential hypertension resulted in lower blood pressure without marked alteration in renal blood flow and sodium homeostasis. These observations, along with studies which have shown that intra-arterial administration of prostaglandin A increased blood flow to the coronary, carotid, femoral, brachial, mesenteric, pulmonary, cutaneous, and renal circulations (Nakano, 1968; Nakano and McCurdy, 1967, 1968; Hauge, et.al., 1967; Lee, 1968) led Lee to suggest that a deficiency of prostaglandin A in the circulating plasma may play a role in the pathogenesis of hypertension (1972a).

Lee has postulated that a deficiency of circulating PGA may alter vasomotor tone in two possible manners. First, a deficiency in arterial PGA levels may result in an increased vasomotor tone due to the lack of the normal vasodilating effect of the prostaglandins on the vasculature. Second, a deficiency of intrarenal and circulating prostaglandin may result in relative cortical ischemia resulting in the release of increased quantities of renin, with the resultant increase in circulating angiotensin II. Either or both of these mechanisms may play a role in the pathogenesis of hypertensive states, in particular the development of essential hypertension.

Materials and Methods

Twenty-three subjects were evaluated as patients in the Yale-New Haven Hospital. Hypertensive medications, diuretics, and potassium supplements were discontinued approximately 14 days prior to evaluation. All patients underwent complete physical examination and laboratory evaluation which included urine analysis and culture, serum blood urea nitrogen, creatinine, sodium, potassium chloride, and bicarbonate, plasma cortisol, twenth-four hour catecholamine, 17-hydroxy steroid, and 17-keto steroid urinary excretion, chest X-ray, EKG, and rapid sequence intravenous pyelography. The diagnosis of essential hypertension was made in those patients with no evidence of adrenal hypertension, parenchymal renal disease, renal artery stenosis or excess catecholamine excretion. The diagnosis of renal artery stenosis was made on the basis of intravenous pyelography and renal arteriography.

Blood was collected in vacutainers containing heparin or EDTA as the anticoagulant and was centrifuged at 4°C (2200 x g) to remove the red cells. Plasma was stored at -20°C until radioimmunoassay for renin activity and prostaglandin A.

Results

ESSENTIAL HYPERTENSION

Table 6 shows the age, sex, blood pressure, daily sodium intake, plasma renin activity,and peripheral prostaglandin A level in the 14 patients with essential hypertension. Figure 13 shows a comparison of these patients' PGA levels with normal humans on low, high, and ad-lib sodium intake diets.

There were 8 male and 6 female subjects; the mean age was 47.7 years, range 24 - 84. Mean PGA levels were 0.60 $\pm$ 0.07 ng/ml, range 0.03 - 0.98. These levels are markedly depressed in comparison to normal humans on ad-lib or sodium restricted diets, p less than 0.001 in each case.

HYPERTENSION SECONDARY TO RENAL ARTERY STENOSIS

Table 7 shows the age, sex, blood pressure, daily sodium intake, plasma renin activity, and prostaglandin A levels in 9 patients with hypertension associated with renal artery stenosis. Prostaglandin A levels were determined in the peripheral plasma in all 9 subjects. In 8 subjects PGA was measured in renal venous blood. The sample designated as ipsalateral represents the renal vein of the kidney with the renal artery stenosis; the contralateral

sample refers to the renal vein from the "normal" kidney.

There were 4 male and 5 male subjects, mean age 40.4 years, range 11 - 65. Peripheral plasma PGA levels were 0.69 ± 0.07 ng/ml (mean ± SEM), range .24 - .96. These levels are markedly depressed in comparison to normal humans on ad-lib or sodium restricted diets (Figure 13), p less than 0.001 in each case.

In all 8 subjects in which renal venous samples were available there were greater levels of PGA in the renal venous plasma from both the ipsalateral and contralateral kidneys in comparison with the peripheral PGA levels. Ipsalateral venous values were 1.23 ± 0.26 ng/ml; or 172.7 ± 31.8% of the peripheral value, p less than 0.05. Contralateral venous values were 1.61 ± 0.30 ng/ml; or 225.7 ± 38.3% of the peripheral value, p less than 0.02. Contralateral venous values were significantly elevated in comparison with the ipsalateral values, p less than 0.002.

CORRELATION OF PLASMA PROSTAGLANDIN A AND RENIN ACTIVITY

Figure 14 shows a comparison of peripheral plasma renin activity and prostaglandin A level. In the studies on the effect of sodium intake on plasma prostaglandin A levels in normal humans, plasma PGA and renin activity were shown to change in parallel fashion in response to changes in dietary sodium intake. In the hypertensive patients studied in this investigation the level of plasma PGA was inappropriately depressed when compared to their plasma renin levels. In 15 of 16 patients there was a significant discrepancy between the prostaglandin A concentration in the venous blood and the simultaneous renin activity.

TABLE 6

PLASMA PROSTAGLANDIN A AND RENIN ACTIVITY IN PATIENTS WITH ESSENTIAL HYPERTENSION

PATIENT	SEX/AGE	SODIUM INTAKE (mEq/24 hours)	PROSTAGLANDIN A (ng/ml)	RENIN ACTIVITY (ng/ml/hour)
1.	F/48	AD-LIB	0.51	0.20
2.	F/58	AD-LIB	0.43	-
3.	M/51	AD-LIB	0.85	-
4.	M/49	AD-LIB	0.84	-
5.	F/42	44	0.59	4.7
6.	F/69	AD-LIB	0.77	-
7.	F/42	AD-LIB	0.68	8.6
8.	M/30	AD-LIB	0.35	2.1
9.	M/25	AD-LIB	0.60	4.7
10.	F/54	AD-LIB	0.79	-
11.	M/25	AD-LIB	0.03	1.8
		10	0.90	6.0
12.	M/84	AD-LIB	0.59	-
13.	M/67	AD-LIB	0.98	-
14.	M/24	AD-LIB	0.32	0.36

TABLE 7

PLASMA PROSTAGLANDIN A AND RENIN ACTIVITY IN PATIENTS WITH RENAL ARTERY STENOSIS

PATIENT	SEX/AGE	SODIUM INTAKE (mEq/24 hours)	Peripheral	Ipsalateral	Contralateral	Peripheral	Ipsalateral	Contralateral
1.	M/19	10	0.76	1.17	1.42	22.5	22.5	15.0
2.	F/11	AD-LIB	0.67	2.51	2.92	3.2	3.9	3.2
3.	F/34	10	0.59	1.04	1.92	27.2	33.7	27.7
4.	M/56	AD-LIB	0.91	1.01	1.38	1.6	2.9	2.5
5.	F/21	AD-LIB	0.60	--	--	3.6	--	--
6.	M/55	10	0.90	1.10	1.48	5.6	15.3	7.1
7.	F/49	10	0.96	2.08	2.56	9.9	10.3	10.4
8.	F/65	10	0.24	0.30	0.31	6.4	21.4	6.3
9.	M/54	10	0.59	0.60	0.86	12.6	32.3	5.8

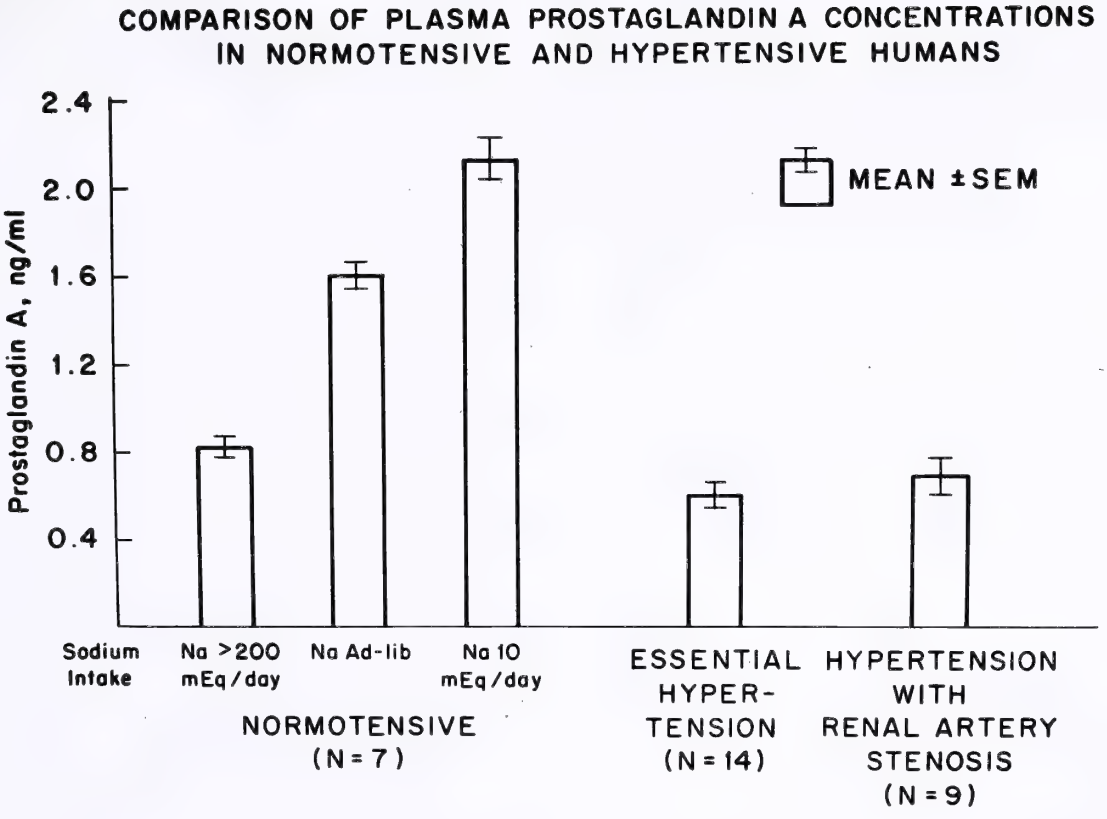


Figure 13. Comparison of plasma prostaglandin A levels in normal humans on high, low, and normal sodium intake diets and patients with essential hypertension or hypertension associated with renal artery stenosis.

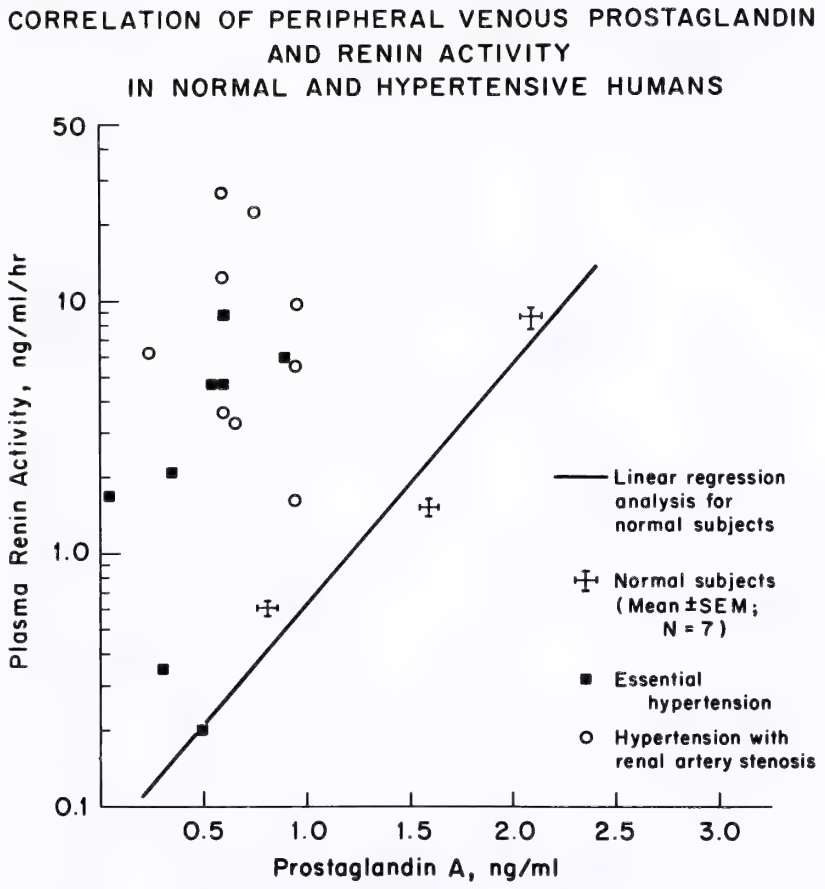


Figure 14. Comparison of plasma renin activity and prostaglandin A concentration in normotensive and hypertensive humans.

DISCUSSION

This study shows that hypertensive humans have a markedly depressed level of circulating prostaglandin A when compared with normal humans on a comparable sodium intake diet. Such decreased levels suggest a severe alteration in the normal mechanisms of prostaglandin synthesis and release in humans with elevated blood pressures secondary to renal artery stenosis or essential hypertension. These measurements confirm the predictions of Lee (1972a) with regard to the levels of circulating PGA in hypertensive humans. The previous observation that hypertensive rats have elevated rather than depressed PGA levels in comparison to normotensive rats presents a paradoxical situation. At present the only explanation available is that the response of the kidney to elevated blood pressure in these two organisms is different.

The reason for the deficient circulating PGA levels in hypertensive humans is not clear at this time. It is possible that such patients have a primary defect in the mechanism regulating prostaglandin synthesis and release thus resulting in lowered levels of circulating PGA, a potent vasodilator. As a result of these decreased levels there may be an increase in blood pressure to abnormal levels. It is also possible that the decrease in circulating PGA represents a secondary deficit; the result of damage by the elevated blood pressure to the mechanism which controls PGA synthesis and release. It is also possible that these patients metabolize PGA at an abnormally high rate. Present information is not sufficient

to distinguish between these three possible explanations for the decrease in circulating prostaglandin A.

In previous studies, it has been shown that the infusion of angiotensin II into the renal artery resulted in the release of prostaglandin-like material into the renal venous effluent (McGiff, et.al., 1970a). Herbaczynaka-Cedro and Vane have shown that the blood pressure raising effect of intravenous angiotensin II is potentiated by prior administration of indomethacin, a potent prostaglandin synthesis inhibitor (1972). This observation suggests that prostaglandins play an important role in the modulation of changes in blood pressure in response to changes in angiotensin production. It is possible that the development of increased blood pressure in patients with essential hypertension or renal artery stenosis reflects not only an increase in vasopressor forces, but also the loss of the normal vasodepressor modulation performed by the prostaglandins, in particular prostaglandin A. The loss of the positive correlation between plasma renin activity and PGA content as shown in Figure 14 suggests that the normal interplay between these two vasotonic substances has been disturbed. It is possible that the loss of prostaglandin A as a vasodilator results in the further elevation of blood pressure in response to alterations in plasma renin and angiotensin levels.

CONCLUSION

THE ROLE OF PROSTAGLANDINS IN SODIUM HOMEOSTASIS

The results of this study with respect to the effect of sodium intake on plasma levels of prostaglandin A in normal humans and spontaneously hypertensive and normotensive Wistar rats do not confirm the basic postulate of Lee's (1972b) theory regarding the role of prostaglandin A as a natriuretic hormone. According to his theory an increase in circulating prostaglandin A is associated with high sodium intake states and a reciprocal decrease in PGA with sodium restriction. Surprisingly our results show that sodium loading produces a marked depression and sodium restriction a marked elevation in circulating PGA. In order to explain these observations the following hypothesis is proposed for the role of prostaglandins in the control of sodium homeostasis (Figure 15).

The observation that infusion of angiotensin II into the renal artery will result in the release of prostaglandin-like material into the renal venous effluent (McGiff, et. al., 1970a) suggests that angiotensin may be the physiologic factor which stimulates the release of prostaglandins linking them to the control of sodium excretion. Under conditions of sodium restriction, renin is released from the juxtaglomerular apparatus of the kidney and acts upon circulating angiotensinogen to result in increased levels of circulating angiotensin I which is then converted to angiotensin II by the converting enzyme of the lung (Mulrow and Goffinet, 1969). Increased angiotensin results in the release

of prostaglandins from the kidney. Enzyme systems recently identified in the kidney (Cammock, 1972) and in human plasma (McDonald-Gibson, McDonald-Gibson, and Greaves, 1972) are capable of converting prostaglandins of the E series to prostaglandins of the A series thereby resulting in an increase in the circulating level of PGA. Previous studies have shown that the major renal prostaglandin is actually prostaglandin E_2 (Lee, et.al., 1967; Crowshaw and Szlyk, 1970; Crowshaw, et.al., 1970). In our own experience with human kidney tissue we found that prostaglandins of the E series made up greater than 60% of the total medullary prostaglandin content, with PGA making up only 15% of the total (Spector, et.al., 1973). It is therefore likely that the enzymatic conversion of PGE to PGA in the plasma is important and contributes to the resultant changes observed in the peripheral circulation. This increased circulating PGA will then antagonize the blood pressure increasing effects of the increased angiotensin II and thus prevent an increase in blood pressure. The studies by Herbaczynska-Cedro and Vane (1972) suggest that this release of prostaglandin is important in modulating the effect of increased angiotensin II concentrations. In their studies animals pretreated with indomethacin showed an increased blood pressure response to intravenous angiotensin II in comparison with the untreated controls. This same prostaglandin synthesis inhibitor had previously been shown to prevent the release of prostaglandins from the kidney in response to angiotensin II infusions (Aiken and Vane, 1971).

It is, therefore, possible that prostaglandins do indeed play an important antihypertensive role in this system.

Studies by Fichman and colleagues (1972) have shown that intravenous infusion of prostaglandin A result in an increase in aldosterone release without measurable changes in ACTH, renin, or serum electrolytes. It is therefore possible that the increase in PGA serves also to stimulate the secretion of aldosterone, which tends to correct the original stimulus, i.e. sodium restriction, by causing increased reabsorption of sodium by the kidney.

Finally there may be an intrarenal role for the prostaglandins in the control of hemodynamics within the kidney. Studies by Hollenberg and colleagues (1970, 1971, 1972a, and 1972b) have shown that there is a decrease in renal cortical blood flow in response to sodium restriction in man. Furthermore, alterations in distribution of intrarenal blood flow observed with sodium restriction are also noted with catecholamine infusion and renal nerve stimulation. Both these stimuli have previously been shown to result in the release of prostaglandin-like material into the renal vein.

We have shown that there are changes in intrarenal concentrations of PGA in response to sodium restriction, parallelling the changes observed in the peripheral plasma. It is possible that the increase in medullary prostaglandins particularly A and E prostaglandins, may result in a medullary vasodilation and thus increased blood flow to the medullary region of the kidney at the expense of the cortical

region. This redistribution of renal blood flow may be important in the reabsorption of sodium by the medullary nephrons. Although there is no evidence that the cortex synthesizes prostaglandins, this region does have a very active enzyme system for prostaglandin metabolism (Nissen and Anderson, 1968 and 1969; Larsson and Anggard, 1972; Anggard, et.al., 1971; Crowshaw, et.al., 1969, 1970). This suggests that intrarenal changes in prostaglandin levels have little or no effect upon the cortical circulation.

In summary, it appears that prostaglandins are indeed "natriuretic" hormones, not in the sense of circulating natriuretic factors as originally postulated by Lee; but rather as intrarenal regulators of blood flow distribution.

This intrarenal regulation of blood flow may be the controlling factor in the degree of sodium reabsorption by the renal nephron population. This hypothesis is schematically portrayed in Figure 15.

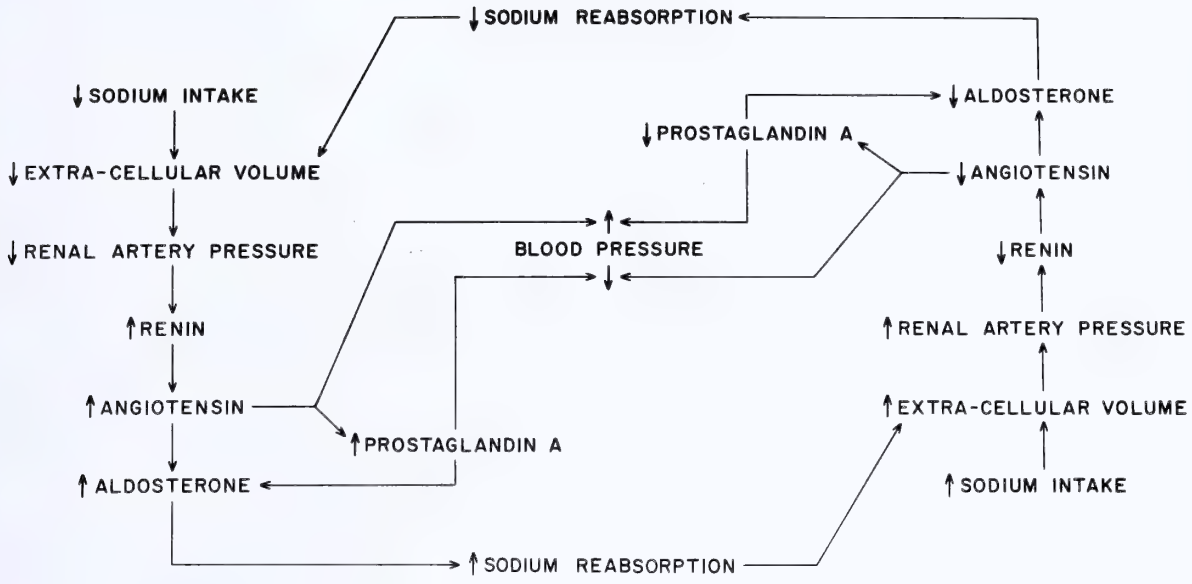


Figure 15. A possible mechanism for the control of prostaglandin A synthesis and release in response to alterations in sodium intake.

PROSTAGLANDIN A AS A CIRCULATING ANTI-HYPERTENSIVE HORMONE

In order to determine the role of prostaglandin A as an anti-hypertensive circulating hormone, plasma PGA levels were determined in both normal and hypertensive humans and rats. Somewhat paradoxically two different observations were made. In the Wistar rat strain, the hypertensive animal had significantly elevated levels of circulating prostaglandin A in comparison with the normotensive Wistar rat. This observation is best ascribed to a compensatory response by the hypertensive animal, the release of a potent vasodilator in an attempt to lower the elevated blood pressure. On the other hand in the human, hypertensive subjects with either essential hypertension or hypertension secondary to renal artery stenosis showed markedly depressed levels of circulating prostaglandin A. It is possible that the development of high blood pressure in some cases of essential hypertension represents the natural course of a deficiency in prostaglandin synthesis and release. Indeed, it may be that essential hypertension is a result of hypoprostaglandinemia, as originally suggested by Lee, and that the restoration of normal plasma levels may be of therapeutic value.

THE KIDNEY AS THE SOURCE OF CIRCULATING PROSTAGLANDIN A

Although many investigators have suggested that the source of circulating prostaglandin A is the kidney it has been impossible until this time to verify or refute this suggestion. This investigation has produced evidence which would seem to substantiate the proposal that the kidney is indeed the source of a major portion of the

circulating prostaglandin A.

As previously noted prostaglandins A_2, E_2 and $F_{2\alpha}$ have been identified in renal medullary tissue (Lee, 1972a) and renal interstitial cells have been shown to produce these prostaglandins when grown in tissue culture (Muirhead, et.al., 1972b). Our studies with rats indicating a close correlation between plasma and renal prostaglandin A concentrations under varying sodium intake diets suggest that the kidney is responsible for the changes seen in these experiments. The marked depression of PGA levels in anephric patients in comparison with PGA levels observed in normal and hypertensive humans suggest that the kidney contributes a major portion of the circulating PGA in human plasma. The observation in patients with renal artery stenosis that renal venous plasma had higher levels of PGA than peripheral plasma also seems to substantiate the suggestion that the kidney does indeed contribute a major portion of the circulating prostaglandin A.

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